

Prevalence of multi-drug resistant *Escherichia Coli* among pregnant women attending Ante-Natal Clinics in Owerri, Imo State, NIGERIA

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ABSTRACT:

The prevalence of *Escherichia coli* (*E.coli*) among pregnant women was studied using urine samples from pregnant women attending antenatal clinics in the two Government hospitals in Owerri; General Hospital, Umuguma Owerri and Federal Medical Centre (FMC) Owerri. One hundred and twenty (120) women (60 from each hospital) were used as subjects. Urine samples (midstream) were collected and analysed aseptically in the Microbiology laboratories of the respective hospitals, within thirty minutes of collection. The results showed that 20 out of the 60 samples collected at General Hospital about i.e. 33.3% harboured *E.coli* while at FMC, 24 samples, i.e. 40% harboured the organism (mean: 36.7%). Antimicrobial susceptibility tests revealed that 32 i.e. 69.6% of the *E.coli* isolated were multi-drug resistant. The highest resistance was to Amoxicillin (100%) followed by Tetracycline (75%) while Ciprofloxacin and Nalidixic acid gave the highest susceptibility (70% each) followed by Pefloxacin (65%). Questionnaires revealed that 80% (16) and 79.2% (19) of those that tested positive to *E.coli* in the respective hospitals were asymptomatic. Result analysis revealed a significant prevalence of resistant *E.coli* among these out-patients who were mostly asymptomatic and unsuspecting carriers. There is therefore need for routine surveillance of these “patients” to ensure that the organism does not cause maternal and child morbidity. The antibiotic susceptibility pattern of the organism should also be ascertained before administering drugs in cases of infection.

Key words: prevalence, *Escherichia coli*, pregnant, women, asymptomatic, resistance

INTRODUCTION

Ante natal patients are ordinarily “healthy patients” i.e. not necessarily presenting any symptom of disease. This though does not mean that they are free of microbial infections. They attend the clinic to ascertain their good health and those of their fetuses from time to time hence they are prone to contact with microorganisms that are of clinical importance.

Escherichia coli is a Gram negative versatile facultative anaerobe which is a normal flora of the colon but when opportuned cause many infections [1, 2, 3, 4] of which Urinary tract infection (UTI) has posed a scourge to pregnant women over the years [1, 2]

The “special” conditions of pregnancy which includes a distended stomach, short urethra, release of specific moisture laden hormones etc may further predispose them to UTIs especially caused by *Escherichia coli* (*E.coli*) [5, 6, 7].

Most types of *E. coli* are harmless and normally inhabit the GI tract; however, some strains have acquired genes that enable them to cause intestinal infection; some types can cause infections such as diarrhea. One type causes travelers' diarrhea. The worst type of *E. coli* (*E. coli* O157:H7) causes bloody diarrhea, and can sometimes cause kidney failure and even death. These problems are most likely to occur in children and in adults with weak immune systems [2, 3, 5]

UTIs are more common in women when compared with men, primarily because of the anatomic

differences of the shorter urethra and its proximity to the vagina and the rectum [8]. However, when pregnant women have a urinary tract infection, they have a higher risk for and increased occurrence of upper tract UTIs when compared with lower tract UTIs [2, 3]. Several physiologic changes occur during pregnancy that cause otherwise healthy women to be more susceptible to serious sequelae from urinary tract infections [8]. The infections can be symptomatic or asymptomatic. Asymptomatic bacteriuria, as the name implies, is a positive urine culture without specific symptoms. Asymptomatic bacteriuria increases the risk for an upper tract UTI, also known as pyelonephritis. Treatment of asymptomatic bacteriuria reduces the risk of a symptomatic infection [9].

Antibiotic resistance i.e. the character of bacteria to withstand the inhibitory or lethal effects of antibiotics, purely demonstrates the versatility of microbes in their struggle for existence [10]. It has been observed that antibiotics susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment [11]. This therefore demands the need for periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities [12].

This study is therefore set to determine the prevalence of the Multidrug resistant forms of *E. coli* among the unassuming pregnant women in Owerri with a view to alerting the women and the entire populace on the presence of such organism and its public health import as well as proffer suggestions for prevention and control of the organism in Owerri and its environs.

MATERIALS AND METHODS

Study location

The research was carried out using antenatal patients of Federal Medical centre, Owerri and General Hospital, Umuguma Owerri which are the two major government hospitals in Owerri metropolis. The informed consent of the hospital management, laboratory scientist and antenatal patients were sought and obtained

Sample and Study population

A total of one hundred and twenty (120) pregnant women (sixty from each hospital) antenatal in both hospitals were used for the study. Mid-stream Urine samples were used for the study. The subjects' consent was obtained for the research.

Materials

Standards materials for microbial isolation, identification and characterization of bacteria were used in the research including consumables and non consumables.

Interviewee and interviewer administered questionnaires were also used.

Sample collection

Each subject was given a sterile wide mouth-screw capped leak-proof container to collect at least 2mls of their mid-stream urine. The names, dates and collection time for each patient was labeled accordingly on the sample container.

Isolation of bacteria

The streaking method of culturing as described by [13] and [14] were used for the isolation; a sterile wire loop was used to pick and inoculate a loopful of each subject's urine onto MacConkey, CLED and blood agar respectively in duplicates. The plates were incubated at 37°C for 18-24 hours. After incubation, the colonies formed were observed and their morphology recorded.

Identification/Characterization of Isolates

Isolates obtained were stained using Gram Staining procedure as described by [13] for preliminary identification of the isolates. To further characterize the isolates, all isolates that were Gram negative rods were subjected to the following biochemical tests as described by [14]; Kigler Iron Agar (KIA) and Motility Indole Urease (MIU) agar test. The results were recorded.

Antimicrobial susceptibility/sensitivity test

Mueller Hinton Agar (MHA) medium and commercial multidisc sensitivity discs were used for the susceptibility test following the Kirby Bauer method

described by [13]. The disc contains the following antibiotics;

Cotrimoxazole 25µg, Nitrofurantoin 200µg, Gentamicin 10µg, Ciprofloxacin 10µg, Ofloxacin 5µg, Augumentin 30µg, Tetracycline 30µg and Amoxicillin 25 µg and Pefloxacin 5µg. Single discs containing Nalidixic acid, were also added alongside the multidisc.

Zones of inhibition were measured and used to extrapolate the level of sensitivity of the isolates to the test antibiotics.

Results are presented using tables and analyzed using simple statistical methods; percentages and averages.

RESULTS

The result of this study is presented in two parts; *Escherichia coli* (*E.coli*) isolation and identification results and Antimicrobial susceptibility test results.

Escherichia coli (*E.coli*) isolation and identification results

Twenty out of the sixty samples (i.e. 33.33%) collected from General Hospital were revealed to harbor *E.coli* while 23 out of the sixty samples collected at FMC were positive for *E.coli*.

On MacConkey and CLED agar, *E.coli* fermented Lactose producing smooth pink and yellow colonies respectively. On blood agar, *E.coli* produced 1-4mm diameter colonies. On Gram staining and viewing under the microscope, they were seen as Gram negative small rods.

The biochemical test results reveal that *E.coli* produced a yellow slope and yellow butt on KIA which indicated fermentation of Lactose. Few drops of Kovac's reagent were added in the test tube containing MIU agar, appearance of red ring indicated indole positivity. It was also found to be motile and Urease positive.

All the questionnaires administered were completed and returned. The analysis of the questionnaires returned revealed that 81.7% (49) and 80% (48) of those tested (i.e. 60 respectively) and 80% (16) and 79.2% (19) that tested positive for the organism at General Hospital, Umuguma Owerri and Federal Medical Centre (FMC) Owerri respectively were asymptomatic of any infection during the time of sample collection.

Antimicrobial susceptibility test results

The antimicrobial susceptibility test results are presented in table 1 below summarized as the percentage effectiveness of the antimicrobials on the test organisms.

TABLE 1: SHOWING PERCENTAGE EFFECTIVENESS OF ANTIBIOTICS ON *E. COLI*

Antibiotic	Symbol	Disc Potency (µg)	No. of Sensitive Isolates	No. of Resistant Isolates	% sensitive Isolates (%)	% Resistant Isolates (%)
Cotrimoxazole	COT	25	21	23	47.7	52.3
Nitrofurantoin	NIT	200	25	19	56.8	43.2
Gentamicin	GEN	10	15	29	34.1	65.9
Ciprofloxacin	CPX	10	31	13	70.4	29.6
Ofloxacin	OFL	5	21	23	47.7	52.3
Augmentin	AUG	30	20	24	45.5	54.5
Tetracycline	TET	30	11	33	25.0	75.0
Amoxicillin	AMX	25	0	44	0	100
Pefloxacin	PEF	5	29	15	65.9	34.1
Ceftriazone	CRO	30	22	22	50.0	50.0
Nalidixic acid	NAL	30	31	13	70.4	29.6

Key: R = Resistant
+ = Susceptible

Discussion and Conclusion

There has been an increasing concern about the prevalence of multi drug resistant *E.coli* strain [3]. From the result obtained above, it is clear that the prevalence of the organism in pregnant women in Owerri < 50. The prevalence of resistant *E.coli* depicts a resistance profile of >50% for all drugs more than half (i.e. 6 out of 10 = 0.6) of the antibiotics tested, some of which form the first line of treatment for most patients in cases of self medication. It is worthy to note that multidrug resistance has serious implication for the empiric therapy of infections caused by *E.coli*.

This study has established therefore that Nalidixic acid, Ciprofloxacin, Pefloxacin and Nitrofurantoin (to which %age susceptibility of the organism was above 50% respectively) are effective drugs that can be used for treatment of *E.coli* infections at their in-use concentrations corroborating [12] while Ceftriazone, Cotrimoxazole, Ofloxacin and Augumentin may not be very effective, Amoxycillin should not be thought of at all for treatment of such infections as all isolates obtained were resistant to it. The study is similar to what was observed by [12] who reported 100% resistance of their *E.coli* isolates to Amoxycillin.

All isolates showed resistance to two or more of the antibiotics tested, this shows that multidrug resistance is truly spreading rapidly among the microbial population. This could be traced to indiscriminate use of antibiotics along with poor hygiene and infection control (risk factor for antibiotic resistance in bacteria) which are highly prevalent in Nigeria and other developing countries [15, 16]. The resistance pattern also show that the resistance could be plasmid mediated as feared by [17] that drug resistance could be plasmid mediated, chromosomal or as a result of drug abuse [18].

Though a high percentage of the women were asymptomatic carriers, it is worthy to note that Asymptomatic bacteriuria increases the risk for an upper tract UTI, also known as pyelonephritis.

Treatment of asymptomatic bacteriuria reduces the risk of a symptomatic infection [19]. Hence the positive women need to be treated. Furthermore, these infections if not identified early and properly treated may lead to infant mortality / morbidity as they may be associated with low birth weight, premature labour, hypertension and/or pre-eclampsia. Maternal amniotitis may also occur [5].

Conclusively, it has been revealed from the findings of this research show that though the prevalence of *E.coli* among pregnant women in Owerri is not high, multidrug resistant *E.coli* dominate the population observed among these women most of who are asymptomatic carriers. This is of serious public health import as the organism can develop infections at the slightest conducive condition and cause harm both to the unassuming pregnant woman and/or her foetus. The high multidrug resistance of *E.coli* observed could be as a result of linear transfer of plasmid (R-factor) from one organism to the other as well as closely linked to irrational use of conventional antibiotics in treating human infections. Special consideration is therefore needed to encourage patients to procure and consume a complete regime of antibiotics. Since antimicrobial resistance patterns are constantly evolving and has become a present global public health problem, there is necessity for constant antimicrobial sensitivity surveillance. There is also need for routine surveillance of these "patients" to ensure that the organism does not cause maternal and child morbidity. The antibiotic susceptibility pattern of the organism should also be ascertained before administering drugs in cases of infection.

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